



# Spatial Autocorrelation Analysis Using MIG-seq Data Indirectly Estimated the Gamete and Larval Dispersal Range of the Blue Coral, *Heliopora coerulea*, Within Reefs

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Spatial autocorrelation analysis is a well-established technique for detecting spatial structures and patterns in ecology. However, compared to inter-population genetic structure, much less studies examined spatial genetic structure (SGS) within a population by means of spatial autocorrelation analysis. More SGS analysis that compares the robustness of genome-wide single nucleotide polymorphisms (SNPs) and traditional population genetic markers in detecting SGS, and direct comparison between the estimated dispersal range based on SGS and the larval dispersal range of corals directly surveyed in the field would be important. In this study, we examined the SGS of a reef-building coral species, *Heliopora coerulea*, in two different reefs (Shiraho and Akaishi) using genome-wide SNPs derived from Multiplexed inter-simple sequence repeat (ISSR) genotyping by sequencing (MIG-seq) analysis and nine microsatellite loci for comparison. Microsatellite data failed to reveal significant spatial patterns when using the same number of samples as MIG-seq, whereas MIG-seq analysis revealed significant spatial autocorrelation patterns up to 750 m in both Shiraho and Akaishi reefs based on the maximum significant distance method. However, detailed spatial genetic analysis using fine-scale distance classes (25–200 m) found an x-intercept of 255–392 m in Shiraho and that of 258–330 m in Akaishi reef. The latter results agreed well with a previously reported direct field observation of larval dispersal, indicating that the larvae of *H. coerulea* settled within a 350 m range in Shiraho reef within one generation. Overall, our results empirically demonstrate that the x-intercept of the spatial correlogram agrees well with the larval dispersal distance that is most frequently found in field observations, and they would be useful for deciding effective conservation management units for maintenance and/or recovery within an ecological time scale.

**Keywords:** spatial autocorrelation analysis, MIG-seq, coral reefs, reef-building coral, larval dispersal

## INTRODUCTION

Coral reefs are highly diverse marine ecosystems, although they are now facing threats such as degradation due to climate change, ocean acidification, frequent outbreaks of corallivore, and other anthropogenic stressors (Wilkinson, 2006). Reef-building corals are the foundation species of coral reef ecosystems that require effective conservation efforts (Carpenter et al., 2008). Although the adults are sessile, reef-building corals release gametes and/or larvae that disperse from their native habitats (Fisk and Harriott, 1990). Because such gamete and larval dispersal results in genetic connectivity among population forming metapopulation structure, estimating the range of gamete and larval dispersal is essential for conservation management (Connolly and Baird, 2010).

Spatial autocorrelation analysis is a well-established technique for assessing spatial dispersal patterns (Epperson, 2005; Schwartz and McKelvey, 2009), but has been overlooked in the field of molecular ecology, especially for benthic marine invertebrates (Billingham and Ayre, 1996; McFadden and Aydin, 1996; Adjeroud and Tsuchiya, 1999; Yund and O'Neil, 2000; Calderón et al., 2007). Compared to intensively studied inter-population genetic structure, much less studies have examined the spatial genetic structure (SGS) of reef-building coral species within a population. Such individual-based SGS within a population have provided important insights into the micro-evolutionary processes and sampling strategies of coral populations (Underwood et al., 2007, 2020; Gorospe and Karl, 2013; Chan et al., 2019; Dubé et al., 2020). However, most of the studies were based on traditional microsatellite loci and to our knowledge no study has used genome-wide single nucleotide polymorphisms (SNPs) and compared the results with the dispersal range directly measured in the field. Theoretically, the detailed genotype information for each sample using genome-wide SNPs would improve the efficiency of detecting significant SGSs, as stochastic error in genotype is one of the challenges in spatial autocorrelation analysis (Slatkin and Arter, 1991).

Owing to the advent of next-generation sequencing (NGS) technology, genome-wide analysis has also become available for non-model organisms. Multiplexed inter-simple sequence repeat (ISSR) genotyping by sequencing (MIG-seq) (Suyama and Matsuki, 2015) is an easy and cost-effective method to obtain a moderate amount of genome-wide SNP data. MIG-seq amplifies putatively neutral ISSR regions, and has proven useful for delimitating species boundaries (Richards et al., 2018; Takata et al., 2019) and for population genetic analysis (Takahashi et al., 2016).

In this study, we used an exhaustive sampling strategy and conducted spatial autocorrelation analysis of two populations of a reef-building coral species, *Heliopora coerulea*, using both traditional microsatellite loci and MIG-seq. *H. coerulea* is a gorgonian coral (Anthozoa, Octocorallia) characterized by an exceptionally hard skeleton. Similar to the primary reef-building corals (Anthozoa, Hexacorallia), this species provides complex habitats for other reef species and thus contributes to sustain high biodiversity in coral reef ecosystems (Zann and Bolton, 1985). In some Indo-Pacific reefs, large populations of *H. coerulea*

are found, and is a dominant coral species (Zann and Bolton, 1985; Takino et al., 2010; Yasuda et al., 2012; Atrigenio et al., 2017). However, the International Union for Conservation of Nature now lists this species as a threatened species because its habitats are degrading, similar to other reef-building coral species (Obura et al., 2008). In this context, identifying the spatial scale of an operational conservation unit – the spatial range of larval and gamete dispersal that occurs in ecological time scales – for *H. coerulea*, is important. *Heliopora coerulea* is a brooding species whose internal fertilization occurs inside female polyps, and almost-competent larvae are released once a year during the summer (Babcock, 1990; Harii et al., 2002; Taninaka et al., 2018). Consistent with the short dispersal duration (Harii et al., 2002), Harii and Kayanne (2003) used settlement tiles in Shiraho reef and directly demonstrated that *H. coerulea* larvae settle within a range of 350 m. Previous population genetic studies performed using microsatellite markers and numerical simulation analyses indicated that larval dispersal among different reef habitats is quite limited (Taninaka et al., 2019); however, one population genetic analysis study reported infrequent long-distance larval dispersal in multiple generations (Yasuda et al., 2014). Strong population genetic differentiation has been observed within a 20 m × 40 km area (Taninaka et al., 2019), indicating that *H. coerulea* may have a finer SGS even within a continuous large population. Individual-based SGS analysis is more useful to detect such a finer scale genetic structure than other population-based genetic methods, and thus would provide more practical larval dispersal estimates for species conservation. Thus, applying SGS analysis for large *H. coerulea* populations would be feasible and beneficial for the conservation of *H. coerulea*. SGS analysis often involves two complementary methods to estimate dispersal range (Peakall et al., 2003; Underwood et al., 2007); one is a conservative estimation that uses the  $x$ -intercept of the spatial correlogram and the other is a more progressive estimation to find the maximum first distance class that is significant. However, no study has ever compared the dispersal distances estimated by alternative SGS methods with direct larval dispersal measurements in the field.

Therefore, this study aimed (i) to examine the SGS of a reef building coral, *H. coerulea*, using traditional microsatellite and genome-wide SNP data for evaluating the effectiveness of using genome-wide SNPs for SGS analysis and then (ii) to compare the dispersal distances estimated by SGS with the direct larval dispersal measurement in the field reported in a previous study (Harii and Kayanne, 2003) to reveal which dispersal estimation method of SGS best matches the direct larval dispersal measurement in the field within ecological time scales.

## MATERIALS AND METHODS

### Sample Collection and Study Sites

All samples from Shiraho reef were collected in 2007 for microsatellite analysis, and the genotyping data were re-used in this study (Yasuda et al., 2010). Samples from Akaishi reef were collected in August 2008 by snorkeling and were preserved in

99.5% ethanol. Genomic DNA samples for microsatellite and MIG-seq analysis were extracted using DNeasy Blood and Tissue kit (Qiagen) from ethanol-preserved tissue samples and were then genotyped as described by Yasuda et al. (2008, 2010). In brief, nine highly polymorphic microsatellite loci were amplified on a PCR thermal cycler, and electrophoresed on 6% Long Ranger sequencing gel (EMC Bioproducts) using SQ-5500E sequencer (Hitachi). Allele sizes were determined using FRAGLYS ver. 3 (Hitachi). First, we used 87 samples from the Shiraho population and 22 samples from the Akaishi population for both MIG-seq and microsatellite analyses (Figure 1). Additional microsatellite analysis was performed by increasing the number of samples (271 for Shiraho and 48 for Akaishi reef). In both the reefs, the maximum depth of the sampling sites during high tide was approximately 3 m and the morphologies of the colonies were either lobate growth form or thick-branching growth form that belongs to *H. coerulea* group in Taninaka et al. (2021).

Both the Shiraho and Akaishi populations are developed within fringing reefs forming micro-atoll structures (most of the colony tops reach the sea level during low tide) located on the southeast (Shiraho) and northeast (Akaishi) coast of Ishigaki Island, southwest Japan. The Shiraho population is one of the largest *Heliopora* populations in the Northern Hemisphere (Harii and Kayanne, 2003). *Heliopora coerulea* in Shiraho is densely, but unevenly distributed on the reef, forming a massive reef pavement behind the crest. Its abundance is high at the center of its distribution in the south (approximately 400 m NS in length with 200 m EW in width and the maximum distance between a pair of samples was found to be 1,288 m). However, the density decreased sharply, forming small patches with colonies spanning several meters to several decimeters in the further north and south of the reef (Figure 1). The Akaishi population is also a large, dense population that was discovered in 2008 (Takino et al., 2010). The Akaishi reef population is 400 m in length (NE–SW) and 30 m in width (NW–SE), and the maximum distance between a pair of samples was 1,948 m (Figure 1). There is a channel crossing the fringing reef crest that exchanges seawater via tidal currents approximately 400 m NE, away from the aggregated population, where we could find a few *H. coerulea* colonies. The Akaishi population is developed near the beach that is 60–80 m away, different from the Shiraho population, which is developed near the reef crest (Takino et al., 2010). The dense colonies of *H. coerulea* in each reef look continuous.

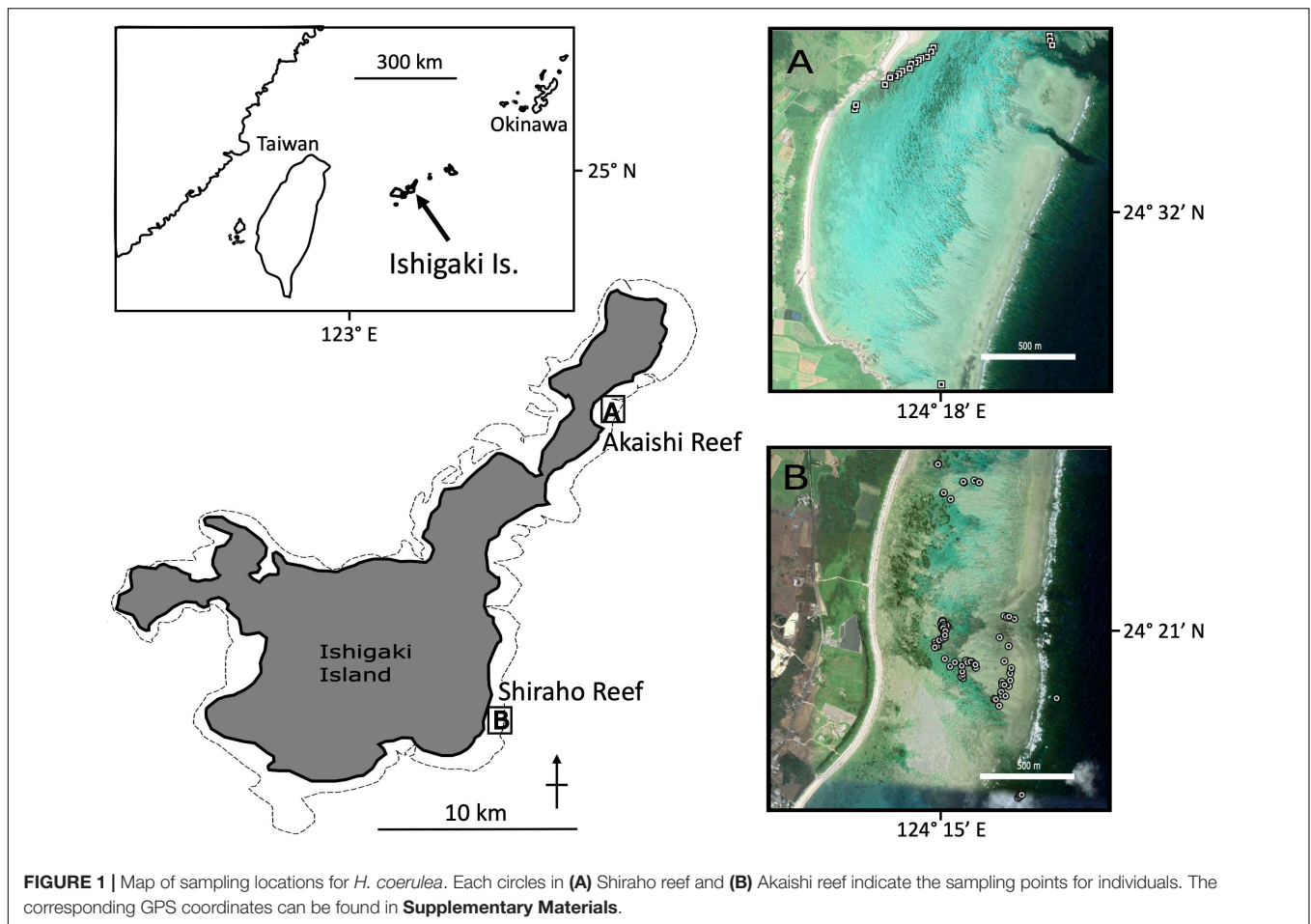
We tried the following two different sampling strategies: random sampling, covering the dense population as evenly as possible, and transect-like linear sampling with relatively regular sampling. In Shiraho reef, we randomly collected samples by covering the whole main dense populations in the south as well as patchily distributed samples in the north. In Akaishi reef, we linearly collected samples from the main population at regular intervals as much as possible, and also collected the patchily-distributed samples near the channel of the reef crest. The raw data for pair-wised geographic distances across samples and microsatellite data sets for Shiraho and Akaishi populations are available in **Supplementary Data 1–3**.

## Single Nucleotide Polymorphisms Detection of MIG-seq

We performed MIG-seq analysis to obtain a relatively large number of SNPs, following the protocol described by Suyama and Matsuki (2015). We used eight pairs of multiplex ISSR primers (MIG-seq primer set 1 in Suyama and Matsuki, 2015). The DNA libraries from each sample with a different index were pooled and sequenced using MiSeq (Sequencing Control Software v2.0.12, Illumina) and MiSeq Reagent v3 150 cycle kit (Illumina). Image analysis and base calling were performed using the real-time analysis software v1.17.21 (Illumina). We then eliminated the low-quality reads and primer sequences from the raw data using FASTX-toolkit (version 0.0.14; fastq\_quality\_filter) (Gordon and Hannon, 2012) with a fastq-quality-filter setting of  $-Q\ 33 -q\ 30 -p\ 40$ . We removed the adapter sequences for the Illumina MiSeq run from both the 5' end (GTCAGATCGGAAGAGCACACGTCTGAACTCCAGTCAC) and the 3' end (CAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAC) using Cutadapt version 2.10 (Martin, 2011) with the  $-e\ 0.05$  option, and we then excluded the short reads less than 80 bp. The quality-filtered sequence data were demultiplexed using Stacks v2.2 (Catchen et al., 2011, 2013). We used the GSTACKS program in Stacks v. 2.2 (Catchen et al., 2013; Rochette and Catchen, 2017) to genotype the individuals using the reference sequence of *H. coerulea* obtained from symbiont-free larvae (Richards et al., 2018). GSTACKS reads all input files simultaneously from the external alignment program, Burrows-WheelerAligner (BWA) version 0.0.17-r118 (Li and Durbin, 2009), and scans the genome for MIG-seq loci using a sliding window method. We used the GSTACKS option ( $-rm\ pcr\ duplicates$ ) to remove PCR duplicates by randomly discarding all but one pair of each set of reads. We used the POPULATIONS program in Stacks v. 2.2 (Catchen et al., 2011) to exclude loci with more than 40% missing data ( $-r\ 0.6$ ). We also changed the  $r$  values (0.9, 0.8, and 0.7) and confirmed that the results did not change for MIG-seq analysis (data not shown here). BayeScan v 2.1 (Narum and Hess, 2011) was used to detect the possible SNPs under natural selection, assuming different species or populations with a default setting for each data set. The SNPs data in the GenAlEx format are available in **Supplementary Data 4**.

## Spatial Autocorrelation Analysis

Multilocus genotypes (MLGs) were determined using the microsatellite dataset. We assumed that the same MLGs found within each population by microsatellite analysis were possible asexually fragmented clones. GenAlEx ver. 6.5 (Peakall and Smouse, 2012) was used to calculate the probability of identity (PI) and spatial autocorrelation analysis. We used two different methods to estimate dispersal range (Peakall et al., 2003; Underwood et al., 2007). One method involved estimation of the dispersal range using the  $x$ -intercept of spatial autocorrelation correlograms, in which the correlation  $r$  is plotted for a given range of distance classes. If larval dispersal is limited and thus gene flow is restricted, the  $r$  value will be significantly positive in the short distance class and will then cross the  $x$ -axis and



become negative (Peakall et al., 2003). The  $x$ -intercept indicates the point at which the random effect of genetic drift can be used to estimate the extent of the positive genetic structure. However, estimation of the  $x$ -intercept is strongly affected by the chosen distance class size and the number of samples within the distance class. In this study, because the dense sampling was within 400 m apart at both sites, we used a distance class size of up to 200 m to estimate the  $x$ -intercept value (so that if any, negative significance could be also detected using a sufficient number of sample pairs). We tested five different distance classes: 25, 50, 100, 150, and 200 m. Another method to estimate dispersal range involves finding the maximum first distant class using multiple distance class plots. Compared to the rather conservative permutation test of the  $x$ -intercept method, this method uses a more powerful permutation, but the range includes weak correlations (low  $r$  value). We used the *Multiple Dclass* option and a 50 m base distance class size for testing 16 distance class sizes.

The analysis superimposes the 95% confidence interval for the null hypothesis of random SGS on the correlogram. The number of permutation tests was set to 9,999 for each analysis. Although we conducted MIG-seq and microsatellite analysis using the same number of samples, we also performed extra spatial autocorrelation analyses using more samples collected

only for the microsatellite data set to test whether the increased number of samples led to significant SGS detection in the microsatellite data (271 Shiraho and 48 Akaishi samples). All data are available in the **Supplementary Material 1–4**.

## RESULTS

Using nine microsatellite markers, we identified possible clones as sample pairs that have exactly the same genotype. Based on the genotyping data using nine microsatellite markers, the PIs were  $5.3 \times 10^{-5}$  for Shiraho and  $4.6 \times 10^{-5}$  for Akaishi reef. In this study, to estimate the range of larval dispersal rather than fragmentation (asexual clonal propagation), we excluded possible clones and used a total of 87 Shiraho samples and 21 Akaishi samples for both MIG-seq and microsatellite analysis. In the microsatellite analysis samples, we used 266 Shiraho samples and 47 Akaishi samples after excluding the possible clones for subsequent analysis.

For MIG-seq analysis, 46,541,760 raw reads with an average of 426,989 reads per sample were obtained from 109 individuals. Low-quality reads were filtered, and 45,269,554 reads with an average of 415,317 reads per sample were obtained. We further excluded index, adapter, and short reads less than

80 bp, and obtained 13,245,206 reads with an average of 121,515 reads. In total, 6,535,144 reads were mapped onto the reference genome, with an average of 59,955 reads per sample. Finally, 2,012 SNPs were obtained from the MIG-seq analysis. BayeScan analysis indicated that no loci were under natural selection ( $q$  values  $>0.05$ ); thus, we used all the loci for the subsequent analysis.

Spatial autocorrelation analysis using a moderate number of loci (2,012 SNPs) based on MIG-seq analysis indicated a significant SGS in both the Shiraho and Akaishi reefs; however, microsatellite analysis using the same number of samples as MIG-seq (87 Shiraho and 21 Akaishi samples) could not reveal a significant SGS in both reefs (Figure 2 and Table 1). By increasing the number of samples, spatial autocorrelation analysis using the microsatellite data (266 samples) in Shiraho reef indicated a significant genetic structure similar to MIG-seq analysis, whereas microsatellite analysis (47 samples, excluding one possible clone) in Akaishi reef could not reveal any significant genetic structure (Figure 2 and Table 1), indicating that MIG-seq analysis is more robust than microsatellite analysis for detecting SGS. Hereafter, we used the SGS data based on MIG-seq analysis.

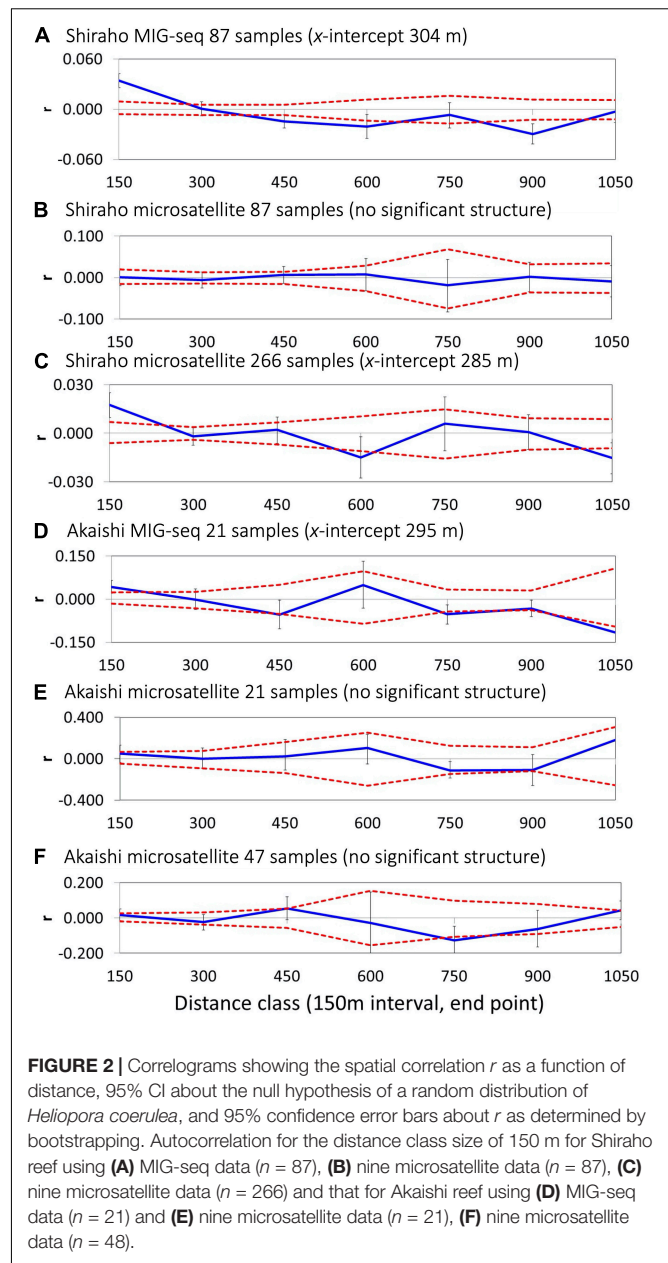
As mentioned above, the estimation of  $x$ -intercept was strongly affected by the selected distance class size and the number of samples within the distance class; using five different distance classes, the  $x$ -intercept values ranged from 255 to 392 m in Shiraho reef and from 241 to 330 m in Akaishi reef for MIG-seq analysis and from 199 to 394 m in Shiraho reef for microsatellite analysis (using 266 samples) (Table 1). The  $x$ -intercept increased as we used a larger distance class, whereas the correlation value  $r$  for the first distance class became smaller as we used a larger distance class (Table 1), indicating a stronger correlation within a smaller geographic range. On the contrary, a finer scale of isolation by distance patterns was detected using a smaller distance class; the shortest first negative significance was detected at 400 m (using 25 and 50 m of distance class) in Shiraho reef and at 275 m (using 25 m of distance class) in Akaishi reef (Table 1).

The maximum first distant class results indicated that the maximum significant distance was up to 750 m in both the Shiraho and Akaishi reefs (Figure 3).

## DISCUSSION

### MIG-seq Data for Spatial Autocorrelation Analysis

Using a moderate number of MIG-seq loci (2,012 SNPs and 87 Shiraho, 21 Akaishi samples), we successfully detected the significant SGS of *H. coerulea* populations both in Shiraho and Akaishi reef. Thus, proximate *H. coerulea* colonies were found to be genetically more alike than more distant colonies. Although spatial autocorrelation analysis is affected by several variances caused by population genetics, stochastic processes, and spatial processes (Slatkin and Arter, 1991), similar patterns of SGS were observed for the two different populations (Shiraho and Akaishi) with different microhabitat environments. This suggests that the positive spatial autocorrelation observed at short



distance classes in MIG-seq analyses supports the small-scale isolation-by-distance patterns due to the limitation of gamete and larval dispersal in *H. coerulea*, as theoretically predicted for species with limited dispersal in the absence of natural selection (Smouse and Peakall, 1999).

In this study, we could directly compare the robustness of microsatellite markers and genome-wide SNP data to detect SGS in sessile shallow-water marine invertebrate species. Microsatellite data using a small number of samples failed to reveal significant structure whereas MIG-seq could successfully reveal significant SGS even when using the same number of samples, thus indicating the robustness of the moderate number of SNPs (2,012 SNPs) in MIG-seq for SGS analysis. It is possible that the resolution of detecting different genetic distances among

**TABLE 1** | The results of spatial autocorrelation of *Heliopora coerulea* and for the analysis with distance class sizes of 25, 50, 100, 150, and 200 m.

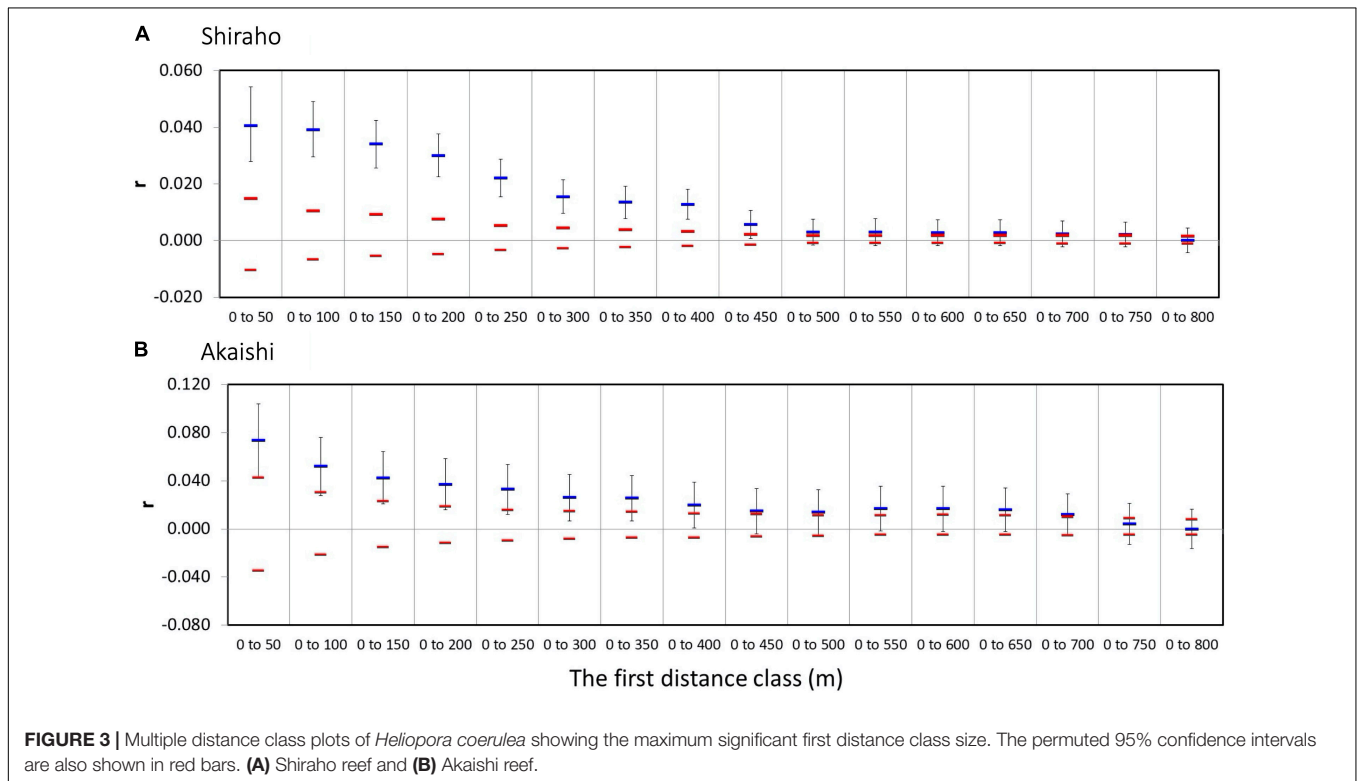
| Population                                       | Shiraho           | Shiraho        | Shiraho           | Akaishi      | Akaishi        | Akaishi        |
|--|-------------------|----------------|-------------------|--------------|----------------|----------------|
| Data used  | MIG-seq           | Microsatellite | Microsatellite    | MIG-seq      | Microsatellite | Microsatellite |
| Number of samples used                           | <b>87</b>         | <b>87</b>      | <b>266</b>        | <b>21</b>    | <b>21</b>      | <b>47</b>      |
| <b>Distance class size = 25 m</b>                |                   |                |                   |              |                |                |
| <i>n</i>   | 165               | 169            | 1083              | 11           | 11             | 83             |
| <i>r</i>   | <b>0.045</b>      | 0.000          | <b>0.011</b>      | <b>0.073</b> | 0.000          | 0.000          |
| U  | 0.020             | 0.046          | 0.017             | 0.063        | 0.212          | 0.076          |
| L  | -0.015            | -0.040         | -0.016            | -0.060       | -0.196         | -0.062         |
| <i>P</i> ( <i>r</i> -rand $\geq$ <i>r</i> -data) | <b>0.001</b>      | 0.168          | 0.091             | <b>0.012</b> | 0.689          | 0.160          |
| Ur   | 0.065             | 0.066          | 0.030             | 0.115        | 0.101          | 0.108          |
| Lr   | 0.026             | -0.024         | -0.007            | 0.029        | -0.197         | -0.038         |
| <i>x</i> -Intercept (m)                          | <b>0</b>          |                | <b>0</b>          | <b>0</b>     | 0              |                |
| First significant negative (m)                   | <b>400</b>        |                |                   | <b>275</b>   |                |                |
| <b>Distance class size = 50 m</b>                |                   |                |                   |              |                |                |
| <i>n</i>   | 310               | 312            | 2252              | 26           | 27             | 158            |
| <i>r</i>   | <b>0.039</b>      | 0.000          | <b>0.012</b>      | <b>0.079</b> | 0.000          | 0.000          |
| U  | 0.015             | 0.033          | 0.012             | 0.043        | 0.131          | 0.051          |
| L  | -0.011            | -0.029         | -0.010            | -0.035       | -0.117         | -0.042         |
| <i>P</i> ( <i>r</i> -rand $\geq$ <i>r</i> -data) | <b>&lt; 0.001</b> | 0.368          | <b>0.026</b>      | <b>0.001</b> | 0.147          | 0.084          |
| Ur   | 0.052             | 0.038          | 0.025             | 0.103        | 0.174          | 0.085          |
| Lr   | 0.026             | -0.030         | 0.000             | 0.056        | -0.040         | -0.017         |
| <i>x</i> -Intercept (m)                          | <b>263</b>        |                | <b>199</b>        | <b>258</b>   |                |                |
| First significant negative (m)                   | <b>400</b>        |                |                   | <b>300</b>   |                |                |
| <b>Distance class size = 100 m</b>               |                   |                |                   |              |                |                |
| <i>n</i>   | 583               | 588            | 3718              | 47           | 49             | 279            |
| <i>r</i>   | <b>0.039</b>      | 0.000          | <b>0.021</b>      | <b>0.052</b> | 0              | <b>0.029</b>   |
| U  | 0.010             | 0.023          | 0.009             | 0.031        | 0.088          | 0.035          |
| L  | -0.007            | -0.019         | -0.008            | -0.022       | -0.074         | -0.028         |
| <i>P</i> ( <i>r</i> -rand $\geq$ <i>r</i> -data) | <b>&lt; 0.001</b> | 0.139          | <b>&lt; 0.001</b> | <b>0.002</b> | 0.193          | <b>0.040</b>   |
| Ur   | 0.049             | 0.036          | 0.031             | 0.076        | 0.127          | 0.068          |
| Lr   | 0.030             | -0.012         | 0.012             | 0.028        | -0.061         | -0.010         |
| <i>x</i> -Intercept (m)                          | <b>280</b>        |                | <b>266</b>        | <b>262</b>   | 279            | <b>166</b>     |
| First significant negative (m)                   | <b>800</b>        |                |                   | <b>500</b>   |                |                |
| <b>Distance class size = 150 m</b>               |                   |                |                   |              |                |                |
| <i>n</i>   | 741               | 803            | 6127              | 67           | 71             | 400            |
| <i>r</i>   | 0.034             | 0              | 0.017             | 0.043        | 0              | 0              |
| U  | 0.009             | 0.020          | 0.007             | 0.023        | 0.062          | 0.025          |
| L  | -0.006            | -0.016         | -0.006            | -0.015       | -0.050         | -0.020         |
| <i>P</i> ( <i>r</i> -rand $\geq$ <i>r</i> -data) | <b>&lt; 0.001</b> | 0.421          | <b>&lt; 0.001</b> | <b>0.002</b> | 0.057          | 0.071          |
| Ur   | 0.043             | 0.022          | 0.025             | 0.065        | 0.132          | 0.051          |
| Lr   | 0.026             | -0.020         | 0.010             | 0.021        | -0.032         | -0.016         |
| <i>x</i> -Intercept (m)                          | <b>304</b>        | 172            | <b>285</b>        | <b>295</b>   | 297            |                |
| First significant negative (m)                   | <b>450</b>        |                |                   | <b>450</b>   |                |                |
| <b>Distance class size = 200 m</b>               |                   |                |                   |              |                |                |
| <i>n</i>   | 936               | 1046           | 9451              | 82           | 88             | 513            |
| <i>r</i>   | <b>0.028</b>      | 0.000          | <b>0.012</b>      | <b>0.039</b> | <b>0.042</b>   | 0.000          |
| U  | 0.008             | 0.016          | 0.006             | 0.018        | 0.046          | 0.019          |
| L  | -0.005            | -0.013         | -0.004            | -0.012       | -0.038         | -0.014         |
| <i>P</i> ( <i>r</i> -rand $\geq$ <i>r</i> -data) | <b>&lt; 0.001</b> | 0.555          | <b>0.001</b>      | <b>0.001</b> | <b>0.032</b>   | 0.128          |
| Ur   | 0.036             | 0.017          | 0.018             | 0.060        | 0.116          | 0.039          |

(Continued)

TABLE 1 | Continued

| Population                     | Shiraho    | Shiraho        | Shiraho        | Akaishi    | Akaishi        | Akaishi        |
|--------------------------------|------------|----------------|----------------|------------|----------------|----------------|
| Data used                      | MIG-seq    | Microsatellite | Microsatellite | MIG-seq    | Microsatellite | Microsatellite |
| Lr                             | 0.021      | -0.020         | 0.006          | 0.017      | -0.034         | -0.019         |
| x-Intercept (m)                | <b>392</b> | 682            | <b>394</b>     | <b>330</b> | <b>628</b>     | 343            |
| First significant negative (m) | <b>800</b> |                |                | <b>600</b> |                |                |

The correlation  $r$  is shown for the first distance class in each analysis.  $n$  is the number of pairwise comparisons for the calculation of  $r$ , upper  $U$  and lower  $L$  bounds for the 95% confidence interval about the null hypothesis of random spatial structure ( $r = 0$ ) and the upper  $U_r$  and lower  $L_r$  about  $r$  as determined by bootstrapping. The probability  $P$  of a one-tailed test for positive autocorrelation, and the estimated  $x$ -intercept of spatial correlogram.  $r$  was converted to zero when  $r$  was not significantly different from zero. First significant negative distances are only shown for MIG-seq data. Significant values are shown in bold face.



individuals was improved by increasing the number of loci (from nine microsatellite loci to 2,012 SNPs). Notably, we could detect significant SGS similar to MIG-seq analysis when using a larger number of samples (266) in the microsatellite analysis. The increased number of samples might have compensated for the small number of loci, and therefore increased the power of SGS detection in the microsatellite analysis. This study demonstrated the robustness of MIG-seq analysis in detecting SGS, which can easily reveal a sufficient number of loci to detect SGS even with a limited number of samples.

## Comparison of SGS Results and Larval Dispersal Based on Direct Field Observation

Estimating the larval dispersal of benthic marine species with pelagic larval duration that occurs on an ecological time scale (within a few generations) is often difficult to extract from

genetic analysis, such as using  $F$ -statistics that include historical gene flow and assume genetic equilibrium (Benzie, 1999). The assignment test is a potential alternative method to estimate dispersal occurring within a few generations if the overall  $F_{ST}$  values are large, such as in terrestrial animals (Berry et al., 2004). Spatial autocorrelation analysis has been proven as a robust method for estimating dispersal range (e.g., Epperson and Li, 1996) and is often applied to plant species (e.g., Smouse and Peakall, 1999), but compared to plant, much less been applied to marine organisms, especially reef-building corals [although there are increasing number of recent references such as Underwood et al. (2007); Thomas et al. (2014), Chan et al. (2019); Dubé et al. (2020), and Underwood et al. (2020)]. This is partly because for marine species, the geographic positions of each individual are relatively laborious to record. In addition, this may also occur because interpretation of resultant distances using SGS ( $x$ -intercept and the maximum significant distance) is rather difficult, and is fundamentally related to the nature of larval

dispersal itself. Even within the same population within a marine species, larval dispersal distance is highly variable and cannot be uniquely determined because of highly fluctuating ocean conditions as well as different dispersal potential among different individuals. In the case of *H. coerulea*, 74% of laboratory-cultured larvae settle within a day, but their competency lasts for up to 30 days (Harii et al., 2002), indicating that *H. coerulea* larvae have infrequent long-distance dispersal potential. The results of SGS in this study could also reflect this nature. The maximum significant (but with weak correlation value) distance found was 750 m for both Shiraho and Akaishi reefs, whereas the  $x$ -intercept using different distance classes ranged from 255 to 392 m in Shiraho reef and from 241 to 330 m in Akaishi reef. By testing five different distance classes (25–200 m), we found the minimum significantly negative spatial range to be 400 m (using the 25 and 50 m distance classes) in Shiraho and 275 m (using the 25 m distance class) in Akaishi reef, implying that most larvae and gametes do not disperse more than 400 m and 275 m in Shiraho and Akaishi reef, respectively. The estimated dispersal range based on the  $x$ -intercept corresponded well with the field observation that larval dispersal in Shiraho reef occurred within a range of 350 m (Harii and Kayanne, 2003), indicating that the  $x$ -intercept of the correlograms agrees well with the ecological larval dispersal range. Such larval dispersal range is essential for maintaining and/or recovering coral populations within an ecological time scale. This empirical demonstration also agreed well with the proposal by Diniz-Filho and De Campos Telles (2002) that the  $x$ -intercept of the spatial correlogram is useful for deciding the management unit for establishing conservation and sampling strategies.

It is also possible that larvae infrequently disperse up to 750 m in Shiraho and Akaishi, as small correlation values imply a maximum significant distance. A previous population genetic study also suggested an infrequent longer larval dispersal of *H. coerulea* (Yasuda et al., 2014). To our knowledge, this is the first study to compare larval dispersal distance measured in the field with the results of SGS analysis in a marine benthic species.

## Larval and Gamete Dispersal of *Heliopora coerulea*

This study demonstrated that the larval and gamete dispersal of *H. coerulea* within well-developed fringing Shiraho and Akaishi reefs occurs at a maximum of 750 m, and the most of the dispersal occurs within 400 m. This indicates that if we are to collect samples from “one population” of *H. coerulea* from Shiraho and Akaishi populations, collection should be within the range of 750 m in diameter. In addition, to determine the conservation unit, a sustainable population within which a sufficient number of larvae can disperse and maintain or replenish the population within an ecological time scale would be less than 400 m in size. The maximum diameter of 750 m indicates that patchily-distributed colonies and dense colonies can be collected within 750 m as one meta-population. Less than 400 m diameter almost corresponded to the size of the dense populations of each studied reef, indicating that gene flow between the dense population and

distant patchy colonies even within the same fringing reef should be conserved separately.

Spatial genetic structure analysis could not distinguish between gamete and larval dispersal. Although the gamete (sperm) dispersal range of *H. coerulea* is still unknown, our data suggest that gamete dispersal may not exceed larval dispersal within a fringing reef, indicating that the dispersal potential of *H. coerulea* sperm is also limited. This provides important insights for the conservation of *H. coerulea*. As *H. coerulea* is typically gonochoric (only one exception was found by Taninaka and Yasuda, 2018), there would be a very low chance of fertilization if the male and female colonies are separated by more than 750 m in Shiraho and Akaishi reefs. Future studies on the spatial distribution of male and female colonies of *H. coerulea* in each population would be important for conservation, as such information is not available currently. The physical environment within well-developed fringing reefs, as in this study, is relatively calmer than that outside the reef crest, where corals are subject to stronger currents as described below. The larval and gamete dispersal distance of *H. coerulea* in a more open environment might thus be larger.

Although we obtained similar results from Shiraho and Akaishi reefs, we cannot generalize these dispersal distance results to other *H. coerulea* populations if each population is subject to different hydrodynamic and environmental conditions. Hydrodynamic surveys have been previously performed on Shiraho reef (e.g., Nakamori et al., 1992; Nadaoka et al., 2001) but not on Akaishi reef. Because of the shallow complex reef topologies associated with several coral microatoll and patches and emergence of well-developed reef crest during low tide, detailed measurement of the current system in Shiraho reef with acceptable accuracy is difficult (Tamura et al., 2007). Field measurement and numerical simulation in Shiraho reef during summer period showed that the current velocity and direction are highly variable depending on tidal cycle; the wave set-up effect and big channels play important role in the current system in Shiraho (Tamura et al., 2007). The study indicated that the overall weak southward tidal-driven current predominates around the dense population of *H. coerulea*. Harii and Kayanne (2003) found no larval disperse and settle in the northern patches in the field. They also found most *H. coerulea* larvae in Shiraho reef dispersed in mid to lower water column where the current velocity is weak (ranging from 1 cm s<sup>-1</sup> during low tide and 3 cm s<sup>-1</sup> during high tide). Given that the larvae of *H. coerulea* can only settle in slower flow velocity in the laboratory experiment (Harii and Kayanne, 2003), it is possible that *H. coerulea* larvae release only when the current velocity is weak regardless of the reef and tend to settle near their parent colonies, similar to our SGS analysis in Shiraho and Akaishi reefs.

Larval dispersal and subsequent SGS might be affected by other environmental factors. Based on the field measurement of the thermal characteristics in Shiraho reef, the distributions of different coral species well agreed with the thermal conditions among different reef micro-habitats (north and south of the moat, reef-flat, and reef-crest) during summer time (Kumagai et al., 2004). The distribution of the dense population of *H. coerulea* corresponded to relatively warm environment in Shiraho reef in



the south (Kumagai et al., 2004). Such study implies the success of larval recruitment and survival, and subsequent SGS depends on the thermal condition of micro-habitats within a fringing reef as well as spatial competition with other coral species. It is also possible that the abundance and distribution of larval predators including butterfly fish (Villanueva and Edwards, 2010) affect dispersal and settlement within the reef.

This study also indicated that if the number of samples are sufficient, the estimated larval and gamete dispersal range found by microsatellite analysis and MIG-seq analysis are comparable and do not depend on the method used for analysis. Other reef-building shallow water brooder coral species whose dispersal range were estimated based on the microsatellite loci had a shorter SGS than that of *H. coerulea*; for instance, a brooding coral *Pocillopora damicornis* (Fan et al., 2006) with a maximum 100 days of competent larvae (Harii et al., 2002) shows only 16 m of dispersal distance based on the *x*-intercept of the correlogram (Tioho et al., 2001; Gorospe and Karl, 2013), whereas another brooding coral, *Seriatopora hystrix* (Fan et al., 2006) with at least 10 days of larval survival period in laboratory experiments (Isomura and Nishihira, 2001), shows a distance of 100 m (based on *x*-intercept) and up to 220 m (based on the multiple distance plots) (Underwood et al., 2007). These species are different from *H. coerulea* in that they are hermaphroditic brooder whose larvae have zooxanthellae (Atoda, 1947, 1951). It is unclear that why such brooding coral species that may have longer larval dispersal potential than *H. coerulea* have shorter dispersal range. It is possible other ecological factors such as abundance of predator of larvae and the environmental factors of the studied areas as discussed above constrain larval dispersal. Further research is needed to determine if and why *H. coerulea* has a relatively longer dispersal range as compared with the two brooding reef-building coral species. On the other hand, broadcast spawner coral species with longer dispersal potential, which does not release competent larvae like brooder corals and in more open environment than fringing reef, had longer dispersal range <12 km (Takata et al., 2021) than *H. coerulea* as revealed by SGS analysis based on genome-wide SNPs like in this study.

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## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: DDBJ (accession: DRA011718), FigShare (doi: 10.6084/m9.figshare.14910546).

## AUTHOR CONTRIBUTIONS

NY conceived the study and collected the samples. HT, TK, HY, and YS performed the MIG-seq analyses (including bioinformatics). DFM, HT, LS, and NY analyzed all the data. DFM and NY drafted and revised the manuscript. All authors have checked and agreed to the final manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.702977/full#supplementary-material>

**Supplementary Data 1** | Pair-wised geographic distances of the samples.

**Supplementary Data 2** | Microsatellite data of Shiraho reef.

**Supplementary Data 3** | Microsatellite data of Akaishi reef.

**Supplementary Data 4** | MIG-seq data in the GenAIEX format.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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